Practical Screening Procedure for Sulfathiazole in Honey

A simple, inexpensive procedure is described for rapidly screening small samples of honey for sulfathiazole (ST), a drug formerly used but not approved in the United States for the prophylactic treatment of American foulbrood disease of bees. The method uses 2 plastic tubes arranged in tandem. The upper tube contains a bed of alumina, which removes some interfering pigments. The lower tube contains a very small bed of anion exchange resin in the HSO_4^- form, which traps the ST. The drug is eventually eluted and detected using the Bratton-Marshall diazotization-coupling reagents. Honey containing 0.1 ppm ST can be readily detected. An optional dye concentration step permits the detection of as little as 25 ppb ST.

In the past, sulfathiazole (ST) was used for treating and preventing the disease of honey bees known as American foulbrood (1, 2). ST was administered to bees as the sodium salt dissolved in a 50% sucrose solution. As a result, honey could become contaminated with ST through this feeding practice. ST, as well as other sulfa drugs, can have an adverse effect on the thyroid in humans. Consequently, ST is not now registered by the Food and Drug Administration for the prevention and control of bee diseases. However, the ready availability of the drug has raised suspicions that it could be used surreptitiously. Honey imported into the United States may also be contaminated with ST. Although there are a number of methods for quantifying ST in honey (3–6), there is no simple procedure for screening a large number of samples, nor is there one that can be used in the field to quickly establish whether there is ST contamination. To remedy this situation, a sensitive, economical, and fairly specific screening method has been developed for ST in honey. This method is noninstrumental, uses no organic solvents, and is sufficiently convenient to be used in the field. It represents an extension of a previously published procedure for determining sulfamethazine in animal feeds (7).

Experimental

Reagents

Use deionized or distilled water throughout. Prepare all solutions in water.

(a) *Sodium nitrite.*—0.12%.

(b) *Ammonium sulfamate.*—0.8%.

(c) *N-1-(Naphthyl)ethylenediamine dihydrochloride (NED).*—(Sigma Chemical Co., St. Louis, MO) 0.8% containing 0.1% ethylenediaminetetraacetic acid.

Solutions (a–c) are conveniently stored in, and dispensed from, drop dispenser bottles (Nalge 2411 Series, A. H. Thomas Co., Philadelphia, PA). The solutions, kept at 4°C when not in use, are usable for up to 2 months.

(d) *Potassium hydrogen sulfate (potassium bisulfate).*—5%.

(e) *Neutral alumina.*—(ALFA Products, Danvers, MA.)

(f) *Ion exchange resin.*—100–200 mesh, chloride from (AG MP-1, Bio-Rad Laboratories, Richmond, CA). Do not substitute.

(g) *Sand.*—Ottawa, 20–30 mesh (Fisher Scientific Co., King of Prussia, PA).

Apparatus

(a) *Transfer pipets, polyethylene.*—Standard pipet (Fisher). Cut ca 1 cm from top of bulb portion and 3 cm from tip portion.

(b) *Pipet tips.*—5 mL (Rainin Instrument Co., Woburn, MA).

(c) *Solid glass beads.*—4 mm (Thomas, No. 5663-L19).

(d) *Melting point capillary tubes with one sealed end.*—Thomas, No. 6418-F10.

(e) *Shell vials.*—¼ dram, 2 mL (Thomas).

(f) *Glass wool, soft.*—Thomas.

Optional Reagents and Equipment

(a) *Color concentrating powder.*—Grind equal weights of Dowex 50 × 12 ion exchange resin, 200–400 mesh (Sigma), and Celite 545 in mortar until uniform in color.

(b) *Disposable glass Pasteur pipets.*—5¾ in. (14.6 cm) (Thomas). Cut ca 6.5 cm from top portion and 2 cm from stem portion.

Preparation of Anion Exchange Resin

AG MP-1 resin (20 g) is stirred magnetically for 1 h with 100 mL $KHSO_4$ solution (pH ~1.1). The suspension is filtered on a 150 mL coarse sintered glass funnel. Excess solution is removed by vacuum application and the resin is washed with water until neutral. Excess water is removed (vacuum), and the resin is transferred to a stoppered vessel and covered with 100 mL water.

Preparation of Lower Tube

Glass wool is rolled into a ball the size of a pea, pushed into the barrel portion of a cut-off transfer pipet, and tamped with a rod so that the fibers are lying flat. The vessel containing the AG MP-1 resin is swirled by hand until the resin is completely suspended, and 0.5 mL suspension is pipetted onto the glass wool plug; the tip of the pipet (1 mL plastic disposable) is positioned just above the plug. After the pipet has drained, 2–3 mm sand is layered on top of the resin.

Preparation of Upper Tube

A 4 mm glass bead is dropped into a 5 mL pipet tip and sand is added so that the tapered portion is just filled. Alumina (1.45–1.50 g) is added and the tip is set piggyback inside the transfer pipet (Figure 1).

Screening Procedure

Honey (1.0–1.2 g) is weighed into a 4 oz paper cup and 5 mL water is added. The contents are stirred with a melting point capillary tube or swirled until the honey dissolves. The solution is poured into the upper tube, and the alumina, when wetted, is stirred with a melting point capillary tube to remove air bubbles. After the solution has percolated through both tubes, 2 mL water is pipetted into the upper tube while washing down the tube walls. After draining, the upper tube is removed and the walls of the lower tube are washed down

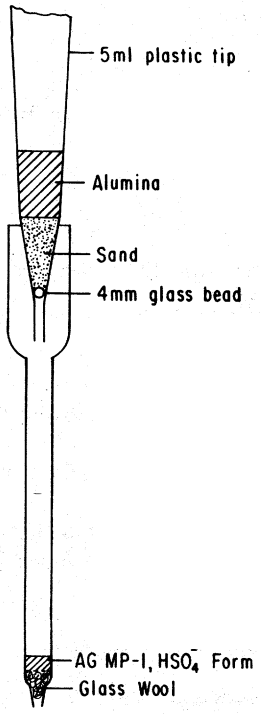

Figure 1. Tandem system for isolating sulfathiazole from honey.

with 1 mL 5% acetic acid. After draining, the lower column is eluted with 0.7 mL 3.5N HCl, and the effluent, which contains the ST, is collected in a shell vial. One drop of $NaNO_2$ solution is added to the effluent and dispersed by shaking by hand for 10 s. One drop of ammonium sulfamate solution is added and the vial is shaken for another 10 s. One drop of NED solution (caution: carcinogen) is added, and the vial is shaken and placed in the dark for 5 min. The development of a pink color constitutes a positive test for ST. When 0.1 μg ST is present in 0.7 mL 3.5N HCl, the color will be clearly evident when the vial is viewed against a white background alongside a reagent blank.

Color Concentration Step (Optional)

If color is not apparent in the reaction vial or if the result is questionable, then the original honey contains 0 or <0.1 ppm ST. To detect color in the vial below the threshold that can be perceived by the eye, the dye can be concentrated on Dowex 50 resin, if desired, enabling as little as 25 ng ST as its colored derivative to be seen. A small piece of glass wool is placed in the upper part of the stem portion of a cut Pasteur pipet, and a small amount of the Dowex 50-Celite powder is added so that, after the powder has been settled by tapping and light tamping, a bed 4–7 mm in height results (Figure 2). The reaction solution is passed over the bed in the dark, and the top 1–2 mm of the bed is examined for a pink or pink-lavender zone. The flow rate of this microcolumn is slow; the solution requires at least 1 h to pass completely through the bed. This step can also be carried out overnight, since the dye is stable on the resin beads for at least 18 h.

Results and Discussion

Screening

A total of 110 samples of honey from at least 20 states in the United States and from 11 other countries were examined. All samples were run in duplicate; one of each duplicate was spiked with 0.1 ppm of the sodium salt of ST. Spiking was performed to ascertain in this initial evaluation of the method whether 0.1 ppm drug could be detected regardless of the type, source, color, or age of the honey. In all but one honey sample, the spike was readily detected. The exceptional sample was an extremely dark honey that gave a yellow color in the acid effluent from the resin that masked the pink color of the Bratton-Marshall dye.

All samples that screened negative or were questionable in solution were subjected to the color concentrating step. Thus, the screened samples were put in one of 3 categories—(I) color visible to the eye in solution, contains ≧0.1 ppm; (II) negative or questionable color in solution but positive on the Dowex 50 color-concentrating bed, contains from 25 ppb to somewhat less than 100 ppb; or (III) negative or questionable in solution and negative on the Dowex 50 column, contains zero or <25 ppb. Of the 110 samples examined, 20 (18%) were in category I, 32 (29%) were in II, and 58 (53%) were in III. The 20 samples in category I ranged in concentrations estimated from 0.1 to 0.7 ppm. One sample contained 3 ppm, but no samples contained between 0.7 and 3 ppm. Estimates for all but the 3 ppm sample were made by comparing the color to artificial, permanent color standards previously prepared for sulfamethazine and described elsewhere (7). The sample containing 3 ppm was estimated spectrophotometrically at 540 nm using a molar absorptivity of 52 500 to calculate its concentration.

It must be pointed out that the samples examined are probably not representative of what is currently available to the public. Some samples were obtained from colonies where sodium sulfathiazole was fed for experimental purposes although this was unknown to us before analysis. Other samples were old and may have been obtained before the registration of sodium sulfathiazole for bee use had lapsed. However, in a limited survey of honey samples more representative of today's commercial market, 6 of 18 samples (33%) were in category I, 3 samples (17%) were in category II, and the remainder (50%) were in category III.

A number of samples were spiked with ST, and recoveries were checked spectrophotometrically. Spike levels of ≧0.5 ppm gave recoveries ≧90%. A spike level of 0.25 ppm gave erratic recoveries averaging about 70%. The erratic and seemingly lower recovery at this level was due to scatter of the unspiked control, which is completely devoid of any dye and can be corrected for in a quantitative method. However, this problem is of no concern in the proposed screening method,

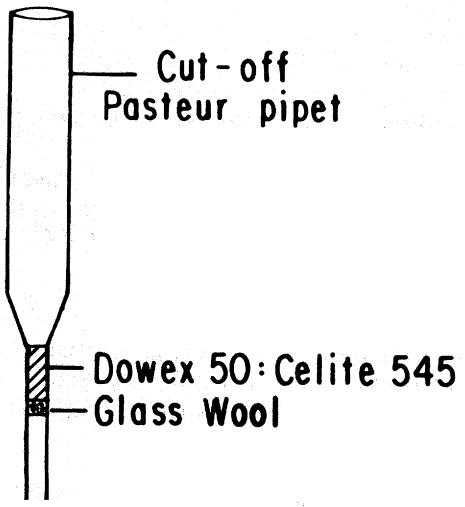

Figure 2. Microcolumn set-up for concentrating Bratton-Marshall dye.

the results of which indicate both a yes-or-no screening decision, and an approximate estimate from the color standards that represents a minimum value not too far from the true value.

Specificity

A number of compounds capable of giving a positive Bratton-Marshall test were similarly screened. The naturally occurring compounds *p*-aminobenzoic acid, anthranilic acid (*o*-aminobenzoic acid), kynurenine (3-anthraniloylalanine), and its decomposition product, *o*-aminoacetophenone, were spiked into honey at the 1.5–2.0 ppm level. All screened negative. Other sulfa drugs were also screened at the 1–2 ppm spike level. Sulfamethazine, sulfamerazine, sulfaguanidine, sulfapyridine, and sulfanilamide were also negative. However, sulfadimethoxine and sulfaquinoxaline at the 1.5 ppm level gave a positive result. These 2 drugs are used in poultry feeding. Sulfanilic acid, a possible breakdown product of sulfa drugs, was negative at the 2 ppm level, as was the procaine salt of penicillin.

The method would thus appear to have good specificity except for the 2 sulfa drugs that are normally not fed to bees. In this regard, it should be pointed out that the screening procedure could be modified, if necessary, so that all sulfa drugs commonly used in animal feeding (sulfamethazine, sulfathiazole, sulfadimethoxine, and sulfaquinoxaline) would give a positive response if present in honey. This would merely require a change in the solution used to equilibrate the resin from $KHSO_4$ to phosphate buffer (pH 7.9) (7). Specificity toward sulfa drugs would have advantages in a screening procedure in the event that sulfa drugs other than ST would be used in bee treatment.

Miscellaneous

A single sample of honey was screened in about 20 min. When the system was properly set up, 10 samples were screened in just under 1¼ h. The samples were first weighed into cups and covered with 5 mL water. The cups were swirled occasionally to facilitate dissolution of the honey. In the interim, the tandem column systems were prepared and placed in a test tube rack (Nalge, 72 hole, No. 5930-0013), which was positioned onto a chromatographic jar or pail to collect the waste effluent. After sample and wash solvents had eluted, the rack was removed and the ends were elevated about 6.5 cm on blocks. The shell vials were then positioned underneath for final eluate collection. The cost of materials is estimated to be ~$0.25 per analysis with disposal of all plasticware and vials.

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